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(54) Title: METHOD FOR THE QUANTIFICATION OF IN VIVO NUCLEIC ACID LEVELS

(57) Abstract: The invention in particular relates to a method for the quantification of *in vivo* RNA from a biological sample comprising the steps of: collecting said biological sample in a tube comprising a compound inhibiting RNA degradation and/or gene induction; forming a precipitate comprising nucleic acids; separating said precipitate from the supernatant; dissolving said precipitate using a buffer, forming a suspension; isolating nucleic acids from said suspension using an automated device; dispersing/distributing a reagent mix for RT-PCR using an automated device; dispersing/distributing the isolated nucleic acids within the dispersed reagent mix using an automated device, and determining the *in vivo* levels of transcripts using the nucleic acid/RT-PCR reagent mix in an automated setup. The present invention also relates to the quantification of DNA from a biological sample. The present invention further elucidates a kit for isolating quantifiable nucleic acids from a biological sample. Applications of the method according to present invention are also disclosed.

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00493

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12N15/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02 00599 A (QIAGEN GMBH ;HOLLAENDER VERA (DE); OELMUELLER UWE (DE); WYRICH RAL) 3 January 2002 (2002-01-03) the whole document	1-17
X	WO 94 18156 A (UNIV IOWA RES FOUND) 18 August 1994 (1994-08-18) claims 1-12; example 1	1-17
X	US 5 906 744 A (CARROLL RICHARD J ET AL) 25 May 1999 (1999-05-25) cited in the application claim 1	5,13,17
X	FR 2 798 673 A (EXONHIT THERAPEUTICS SA) 23 March 2001 (2001-03-23) page 11 -page 12; claims 1-5	20-22
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

7 November 2003

Date of mailing of the international search report

19 DEC 2003

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 49083 A (UROCOR INC) 30 September 1999 (1999-09-30)  claims 1-5; examples 5-7 ----	20-22, 25,27, 30-36
X	WO 99 57130 A (GENE LOGIC INC ;PRASHAR YATINDRA (US); WEISSMAN SHERMAN (US)) 11 November 1999 (1999-11-11) claim 1 ----	23,26
X	WO 94 28123 A (ONTARIO CANCER INST ;THOMPSON CRAIG BERNIE (US)) 8 December 1994 (1994-12-08) claim 7 ----	23,26
P,X	WO 02 086113 A (MOFFAT MIRIAM FLEUR ;ALLEN MAXINE (GB); LENCH NICK (GB); COOKSON W) 31 October 2002 (2002-10-31) the whole document ----	20-22, 25,27, 30-36
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 16 November 2001 (2001-11-16) RAINEN LYNNE ET AL: "Stabilization of expression profiles of genes associated with the inflammatory response in post-phlebotomy whole blood using the PAXgene™ blood RNA system" Database accession no. PREV200200151792 XP002260618 abstract & BLOOD, vol. 98, no. 11 Part 2, 16 November 2001 (2001-11-16), pages 108b-109b, 43rd Annual Meeting of the American Society of Hematology, Part 2;Orlando, Florida, USA; December 07-11, 2001 ISSN: 0006-4971  ----- -/--	20-22

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00493

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE BIOSIS 'Online!  BIOSCIENCES INFORMATION SERVICE,  PHILADELPHIA, PA, US;  16 November 2001 (2001-11-16)  MUELLER MARTIN C ET AL: "Improvement of  molecular monitoring of residual disease  in leukemias by bedside RNA stabilization  with the PAXgene blood RNA system"  Database accession no. PREV200200129905  XP002260619  abstract  &amp; BLOOD,  vol. 98, no. 11 Part 1,  16 November 2001 (2001-11-16), pages  111a-112a,  43rd Annual Meeting of the American  Society of Hematology, Part 1;Orlando,  Florida, USA; December 07-11, 2001  ISSN: 0006-4971</p> <p>-----</p>	20-22

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 03/00493

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 28, 29  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 24  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
1-17, 20-36
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 30-36 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the in vitro detection of the change in in vivo levels of immuno-related transcripts.

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Continuation of Box I.1

Claims Nos.: 28, 29

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

Claims Nos.: 24

Present claim 24 relates to a compound defined by reference to a desirable characteristic or property, namely being identifiable by a method according to claim 23.

The claims cover all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, the no search has been carried out for claim 24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-17

Methods and kits for isolating quantifiable in vivo RNA from a biological sample.

Invention 2: claims 18,19

Methods and kits for isolating quantifiable in vivo DNA from a biological sample.

Invention 3: claims 20-36

Methods and kits for the diagnosis/ prognosis/ monitoring of a clinical status affecting the immune system, for the screening for compounds modulating the clinical status affecting the immune system, and uses thereof.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00493

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International Application No

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